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ORIGINAL RESEARCH

Immobilization of a cellulose carbamate-type chiral selector onto silica gel by alkyne-azide *click chemistry* for the preparation of chiral stationary chromatography phases

Cuong Viet Bui · Thomas Rosenau · Hubert Hettegger

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Abstract A new synthesis strategy for the preparation of cellulose derivative-based chiral selectors and the subsequent mild immobilization onto pre-functionalized silica gel are introduced, utilizing Cu(I)-catalyzed alkyne-azide *Huisgen* cycloaddition ("*click*") chemistry. A cellulose 3,5-dimethylphenyl carbamate derivative carrying propynyl carbamate groups was prepared by a combination of carbonate aminolysis and isocyanate chemistry. For immobilization, 3-azidopropyl-functionalized silica gel as an inert carrier was used, synthesized *via* a 3-chloropropyl intermediate. The chiral selector, as well as the inorganic/organic hybrid materials (silica gel/chiral selector), were comprehensively characterized by ATR-FTIR, solid-state ^{13}C and ^{29}Si NMR, liquid-state NMR, GPC, TGA, and elemental analysis. The enantioseparation performance of the immobilized-type chiral stationary phase was evaluated by HPLC with a set of representative chiral test analytes and different eluents and compared to a respective coated-type (=non-covalently bound) chiral stationary phase carrying the same selector quality and quantity on the same silica gel matrix. The immobilization did not adversely affect the chiral separation performance; on the contrary, in some chromatographic separations the immobilized-type chiral stationary phase surprisingly even surpassed the coated reference material.

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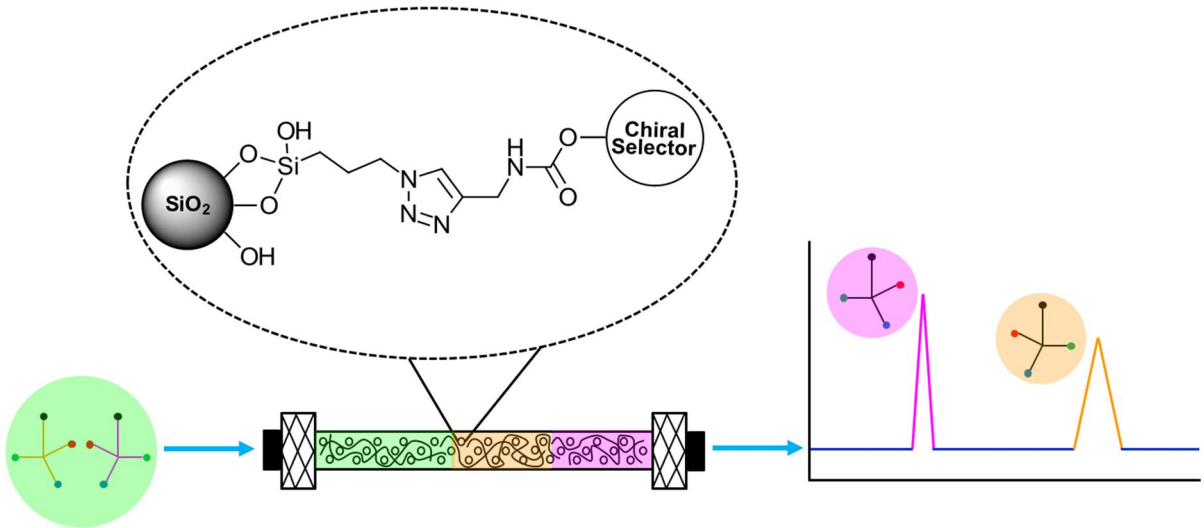
Graphical abstract



Keywords Carbonate aminolysis · Cellulose derivatization · Chiral chromatography · Chiral stationary phase · Click chemistry · Enantioseparation · Inorganic/organic hybrid materials

Introduction

Cellulose is a well-known raw material in many industrial applications, *e.g.*, paper, board, fiber production, and various cellulose derivatives. Besides this large-scale (and comparably low value) utilization, there are also various niche applications, in which cellulose is processed into specialized high-tech and high-value materials on a lower scale. Cellulose derivative-based chiral selectors (CSs), key components in chromatographic columns for enantioseparation applications in liquid chromatography (besides amylose derivatives), are one example. Natural cellulose—which is *per se* chiral and enantiomerically pure—is used as a CS, however, the enantioseparation performance does not meet the requirements of analytical and preparative scale separation. Therefore, cellulose needs to be derivatized to increase the overall enantioseparation performance (Li et al. 2020; Wang et al. 2021a). The enantioseparation ability of cellulose derivatives was originally investigated by Lüttringhaus and co-workers in 1967 when they applied cellulose triacetate for the enantioseparation of 4.5.6.7-dibenzo-1.2-dithiacyclooctadien in liquid chromatography (Lüttringhaus et al. 1967). A large number of CSs based on phenyl carbamates and/or benzoate esters of cellulose was introduced and has been commercialized over the last 40–50 years (Minguillón et al. 1996; Fanali et al. 2019; Onishi et al. 2022). Polysaccharide derivative-based CSs based on cellulose and amylose can nowadays separate about 90% of the tested racemic compounds and enantiomeric mixtures (Ikai and Okamoto 2009; Shen and Okamoto 2016; Yin et al. 2019). Important fields of enantioseparation are, *inter alia*, stereoselective synthesis (Chebrouk et al. 2019; D'Orazio 2020; Shi et al. 2020), the analysis of enantiomers in food products (Fanali et al. 2019; Alvarez-Rivera et al. 2020), fungicides (Ying et al. 2009; Ruiz-Rodríguez et al. 2015; Wang et al. 2021b), herbicides (Martín-Biosca et al. 2001; Lao and Gan 2006; Jin et al. 2010), insecticides (Zhang et al. 2016; Zhao et al. 2019), pesticides (Carrão et al. 2019; Merino et al. 2019; Li et al. 2022), and—very importantly—pharmaceutical products (Mukherjee and Bera 2012; Yaling and Alex 2013; Singh et al. 2020).

High-performance liquid chromatography (HPLC) is one of the most powerful techniques for direct enantioseparation on both analytical and preparative scales (Ahuja 1997; Subramanian 2008). In the case of HPLC (including its variant supercritical fluid chromatography, SFC), a chiral stationary phase

(CSP) composed of silica gel as an inert, mechanically stable carrier is combined with an enantiopure CS and used for the direct separation of enantiomers *via* the formation of differently stable transient diastereomeric complexes between CS and the respective enantiomers (Davankov 1997; Lämmerhofer 2010). The conventional method for the preparation of CSPs is the physical coating of CSs onto (often pre-functionalized) silica gel. Pioneering work in this field has been performed by Okamoto et al. since the 1980s (Okamoto et al. 1984b), from which many coated-type CSPs resulted that have been successfully commercialized and are still available (Okamoto et al. 1984a, 1986; Okamoto and Kaida 1994; Yamamoto and Okamoto 2004; Yin et al. 2019; Bui et al. 2021). However, the high solubility and the swelling behavior of polysaccharide derivative-based CSs limits their use, with a number of organic solvents or mobile-phase components, such as chloroform, tetrahydrofuran, ethyl acetate, acetone, etc., being rather incompatible. This leaves comparably little room for method optimization and development due to the limited number of applicable solvents, narrowing down the choices of chromatographic mobile phases (Minguillón et al. 1996; Yashima et al. 1998; Francotte 2001; Ikai et al. 2007b; Padró and Keunchkarian 2018; Chankvetadze 2019; Yin et al. 2019; Wang et al. 2021a; Onishi et al. 2022).

Immobilization of CSPs, *i.e.*, covalent chemical linkage of cellulose derivative-based CSs onto pre-functionalized silica gel, is thus the most obvious and suitable option to overcome the shortcomings of its "coated-only" counterparts, and their inherent problems with the dissolution of the CS in strong eluents, resulting column bleeding, and, in the worst case, destruction of the column material (Tang et al. 2011; Padró and Keunchkarian 2018; Chankvetadze 2019; Fernandes et al. 2021; Onishi et al. 2022). One of the first immobilized, chemically robust CSPs were introduced by Okamoto et al. as well, when they linked cellulose 3,5-dimethylphenyl carbamate- and 3,5-dichlorophenyl carbamate-type selectors onto 3-aminopropyl-functionalized silica gel by crosslinking with diisocyanates (Okamoto et al. 1987). However, immobilized CSPs are often characterized by a somewhat lower enantiomer recognition capacity compared to their coated-type counterparts, due to reduced flexibility of the CS, especially when having many anchor points to the supporting matrix along the polymer chain (Chang et al. 2018).

Several methods to immobilize CSs onto pre-functionalized silica gels have been introduced: linkage/crosslinking with diisocyanates (Chen et al. 2002, 2003a, b, c; Tang et al. 2011), condensation of alkoxysilyl groups (Ikai et al. 2006, 2007a; Tang et al. 2010a, b; Shen et al. 2012; Yu et al. 2020), polymerization with vinyl groups (Kimata et al. 1993; Minguillón et al. 1996; Franco et al. 1998; Garcés et al. 2003; Kubota et al. 2004; Chen et al. 2006; Chen and Hsieh 2011; Bae et al. 2011), epoxy groups (Chankvetadze et al. 2004; Dong et al. 2008), amidation (Miaomiao et al. 2017), thiol-ene addition (Huang et al. 2014; Yao et al. 2016; Yin et al. 2019; Li et al. 2019; Zhou et al. 2020), and Staudinger ligation (Zhang et al. 2007; Peng et al. 2012; Tan et al. 2014; Silva et al. 2017; Lin et al. 2018). As self-crosslinking reactions are often hard to control, the immobilization of polysaccharide-type CSs by diisocyanate, alkoxysilyl, and vinyl group methods is challenging, and the corresponding immobilized-type CSPs have typically a lower chiral discrimination capacity in comparison to the respective coated CSPs. Further possible drawbacks associated with the above-mentioned immobilization methods are the decrease in chiral discrimination capacity, low immobilization efficiency, low stability of the linkage between CS and pre-functionalized silica gel, as well as time-consuming and complex chemical processes and reactions in the preparation of the immobilized-type CSPs (Kubota et al. 2004).

The jargon term *click chemistry* introduced by Sharpless and colleagues in 2001 (Kolb et al. 2001) is usually referring to fast, neat, and easy-to-handle chemical reactions, which are efficiently carried out under mild conditions with almost quantitative yield. The most well-known example is the Cu(I)-catalyzed alkyne-azide *Huisgen* cycloaddition, in which stable, mostly 1,4-disubstituted 1,2,3-triazoles are formed (Rostovtsev et al. 2002; Aflak et al. 2019). Nowadays, this reaction is applied in many technological areas, such as drug discovery (Totobenazara and Burke 2015), material sciences, chemical biology (Lauria et al. 2014; Burke and Marques 2015), cell labeling (Sletten and Bertozzi 2011; Burke and Marques 2015), and peptidomimetic chemistry (Pedersen and Abell 2011; He et al. 2014; Solum et al. 2014; Pingaew et al. 2014), to name but a few. The

alkyne-azide cycloaddition reaction has already been used for the immobilization of *Cinchona* derivatives (bearing alkyne groups) onto 3-azidopropyl-functionalized silica gel to prepare anion-exchange-type CSPs (Kacprzak et al. 2006, 2011; Kacprzak and Lindner 2011; Hettegger et al. 2014). In cellulose chemistry, this type of *click chemistry* is also well-developed. The polysaccharide can either act as the alkyne donor (for instance after etherification with propargyl halides) or as the azido-type acceptor (*e.g.*, after tosylation and exchange for azide at C6 or after heterogeneous functionalization with (3-azidopropyl) trialkoxysilanes) (Liebert et al. 2006; Hettegger et al. 2015a, 2015b, 2016).

In this account, we would like to communicate another approach, a one-pot, three-step synthesis of a cellulose 3,5-dimethylphenyl carbamate-type selector carrying propynyl carbamate groups for *click* immobilization. This is achieved by a combination of carbonate aminolysis and isocyanate chemistry. The obtained cellulose derivatives were subsequently immobilized onto 3-azidopropyl-functionalized silica gel by the mentioned alkyne-azide *Huisgen* cycloaddition chemistry to give chemically and physically robust CSPs. All compounds and materials were comprehensively analytically characterized by FTIR, solid-state ^{13}C and ^{29}Si NMR, 2D liquid-state NMR, GPC, and elemental analysis (EA). The loading amount of the CS on the silica matrix (*i.e.*, the organic content of the immobilized CSP) was determined by both EA and TGA. Eventually, the enantioseparation performance of the immobilized-type CSP was evaluated and compared to a coated-type CSP prepared with the same CS type and CS amount.

Materials and methods

Materials

Microcrystalline cellulose (Avicel® PH-101), *N*,*N*-diisopropylethylamine (> 99%), *p*-toluenesulfonic acid (98.5%), tetra-*n*-butylammonium iodide (99%), and *p*-cymene (99%) were purchased from Sigma-Aldrich (Schnelldorf, Germany). Microcrystalline cellulose was dried at 40 °C in a vacuum oven for at least two days before use. 3,5-Dimethylphenyl isocyanate (> 98%), phenyl chloroformate (> 98%), (3-chloropropyl)trimethoxysilane (> 97%), and NaN_3 (> 99%) were purchased from TCI Europe N.V. (Zwijndrecht, Belgium). Organic solvents, such as *N*,*N*-dimethylacetamide (DMAc), *N*,*N*-dimethylformamide (DMF), tetrahydrofuran (THF), acetonitrile (MeCN), and pyridine, were all reagent grade and dried over 3 Å molecular sieves (Sigma-Aldrich) for at least three days before use. Ethanol (EtOH) and methanol (MeOH) for precipitation and washing were of technical grade and obtained from Carl Roth GmbH + Co. KG (Karlsruhe, Germany) or Fisher Scientific (Vienna, Austria). Silica gel (NUCLEOSIL® 1000–7, 7 µm, 1000 Å, 25 m^2/g by BET) was purchased from Bruckner Analysentechnik GmbH (Linz, Austria). Rhodamine B propargyl ester was synthesized according to a published protocol (Hettegger et al. 2016). Empty stainless steel HPLC columns (150 × 4 mm, i.d.) and column hardware were purchased from Bischoff Analysentechnik u. -geräte GmbH (Leonberg, Germany). The commercial chiral analytes, 2-phenylcyclohexanone (> 98%, **a**), benzoin (> 98%, **b**), and Pirkle's alcohol (> 99%, **d**), were purchased from TCI Europe N.V. (Zwijndrecht, Belgium). Flavanone (98%, **c**), *trans*-stilbene oxide (98%, **e**), and Tröger's base (98%, **f**) were obtained from Sigma-Aldrich (Schnelldorf, Germany). Mandelic acid derivatives (**g–j**), 1-methoxy-2-(1-methoxy-3-phenylpropyl) benzene (**k**), and 1-(*o*-hydroxyphenyl)-3-phenyl-1-propanol (**l**) were synthesized according to standard procedures. The HPLC solvents *n*-hexane (95%, *n*-hex), 2-propanol (99.9%, IPA), tetrahydrofuran (> 99.9%, THF), and chloroform (99.8%) were obtained from Fisher Scientific. Formic acid (97.5–98.5%, FA) and diethylamine (99.5%, DEA) as modifiers for the HPLC solvents were purchased from Sigma-Aldrich (Schnelldorf, Germany).

Instrumentation

Elemental analyses were performed with a EURO EA 3000 CHNS-O instrument (HEKAtech, Wegberg, Germany), and halide contents were determined by argentometry, both at the microanalytical laboratory of the University of Vienna, Austria. Thermogravimetric analysis (TGA) was carried out with a TG 209 F1 Iris thermo-microbalance (Netzsch GmbH & Co. KG, Selb, Germany), with a dried sample mass of 10–15 mg, an oxidizing atmosphere (N_2:O_2 = 4:1, v/v), a flow rate of 20 mL/min and a T gradient of 10 °C/min. All measurements were done in triplicate.

Proteus software was used for data processing and evaluation of TGA results. Solid-state ^{13}C CP/MAS (12 kHz), ^{29}Si CP/MAS (8 kHz), and HSQC liquid-state NMR experiments were carried out on Avance III HD and Avance II 400 instruments (Bruker, Rheinstetten, Germany) with a resonance frequency of 400.13 MHz for ^{1}H, 100.67 MHz for ^{13}C and 79.53 MHz for ^{29}Si. NMR spectra were evaluated using both TopSpin 3.6.2 and ACD/NMR Processor Academic Edition 12.01. Chemical shifts (δ) are given in ppm. ATR-FTIR spectra were recorded on a Frontier IR single-range spectrometer (PerkinElmer, Waltham, Massachusetts, US) equipped with a diamond/ZnSe crystal, $LiTaO_3$ detector, and KBr windows. Data processing was performed with SpectraGryph software (version v1.2.15). GPC analyses were performed according to standard procedures. For details about GPC procedures see (Henniges et al. 2011; Jusner et al. 2022). An Agilent Technologies, Inc. (Santa Clara, CA, USA) 1100 HPLC apparatus equipped with a degasser (G1322A), quaternary pump (G1311A), autosampler (G1313A), thermostatted column compartment (G1316A), and DAD (G1315A) was used to evaluate the enantioseparation performance of the chiral columns. OpenLab CDS software (Agilent) was used for chromatography data processing and evaluation.

Synthesis

One-pot, three-step synthesis of the chiral selector (CS)

(1) *Oxycarbonylation*: Microcrystalline cellulose (1.5 g) was immersed in anhydrous DMAc (45 mL) and then heated at 120 °C for 2 h under a dry nitrogen atmosphere during vigorous stirring. Cellulose degradation during that heating period was considered to be tolerable (Potthast et al. 2003). Anhydrous LiCl (2.7 g) was added slowly after cooling down to < 90 °C. The mixture was allowed to stir at room temperature (RT) until a clear solution was formed. Anhydrous pyridine (4.5 mL) was added slowly and the homogeneous solution was cooled down to 0 °C by an ice/water bath. Phenyl chloroformate (0.36 mL, 0.3 molar equivalents with respect to the glucopyranose repeating unit of cellulose) was added dropwise during 10 min and the mixture was stirred for 12 h at RT.

(2) *Aminolysis*: Anhydrous DMF (4.5 mL) was added to the reaction mixture, followed by the addition of propargylamine (0.78 mL, 5 molar equivalents with respect to phenyl chloroformate), and the mixture was stirred at 40 °C for 12 h.

(3) *Carbamoylation*: Anhydrous pyridine (15 mL) was slowly added to the reaction mixture, followed by 3,5-dimethylphenyl isocyanate (5.5 mL, 4.5 molar equivalents rel. to the glucopyranose units of cellulose). The mixture was stirred at 80 °C for 18 h and subsequently cooled down to RT. A large excess of distilled water was used to precipitate the crude **CS**, which was collected by vacuum filtration, and washed with a large excess of distilled water and EtOH (two times each). The crude **CS** was dried at 40 °C in a vacuum oven for two days and re-dissolved in acetone (375 mL). Residual LiCl was separated by vacuum filtration through a sintered frit. Acetone was largely removed by rotary evaporation under reduced pressure and the **CS** was precipitated in EtOH, collected by vacuum filtration, washed with a large excess of EtOH and distilled water (two times each), and dried at 40 °C in a vacuum oven for two days. Yield: 4.85 g, 87 wt%.

Pre-modification of silica gel (AzPS)

Protocols reported by (Kacprzak et al. 2006) and (Hettegger et al. 2014) were adapted to synthesize 3-azidopropyl-functionalized silica gel (**AzPS**) with modifications.

(1) *3-Chloropropyl-functionalized silica gel*: Silica gel (10 g) and *p*-toluenesulfonic acid (20 mg) as a catalyst were immersed in toluene (200 mL) and mechanically stirred. The suspension was dried by azeotropic distillation under a dry nitrogen atmosphere. After distilling off about half of the volume of toluene, the temperature of the suspension was reduced to 80 °C, and (3-chloropropyl) trimethoxysilane (4.6 mL, 25.2 mmol) was added dropwise to the mixture during 15 min. The reaction mixture was mechanically stirred for 48 h. The suspension was then cooled down to RT and the formed **ClPS** was collected by vacuum fil-

tration through a sintered glass frit (DURAN®, porosity 4), washed with toluene (100 mL), and with MeOH (100 mL, two times each). **ClPS** was subsequently re-immersed in toluene (100 mL) and mechanically stirred at 80 °C for 2 h. The suspension was then cooled down to RT and **ClPS** was again collected by vacuum filtration, washed with toluene (100 mL), MeOH (100 mL), and distilled water (200 mL, two times each), and dried at 40 °C in a vacuum oven for two days. Yield: 10.0 g.

(2) *3-Azidopropyl-functionalized silica gel*: **ClPS** (10 g), tetra-*n*-butylammonium iodide (60 mg) as a catalyst, and NaN_3 (4.9 g, 75.4 mmol) were dispersed in DMSO (100 mL) and the suspension was mechanically stirred at 80 °C for 72 h under a dry nitrogen atmosphere and then cooled to RT. The obtained **AzPS** was collected by vacuum filtration through a sintered glass frit (DURAN®, porosity 4), washed with H_2O (500 mL) and MeOH (250 mL, two times each), and dried at 40 °C in a vacuum oven for two days. Yield: 10.0 g.

Preparation of immobilized CS (CSP1)

The above **CS** (1 g) was dissolved and stirred in THF (100 mL) in a Schott DURAN® bottle. **AzPS** (3 g) was added and the suspension was shaken on an overhead shaker for 24 h. A catalyst solution was prepared by dissolving CuI (17 mg) in MeCN (10 mL). *N*,*N*-Diisopropylethylamine (1 mL) was added to the **CS / AzPS** mixture in THF, followed by the CuI solution. The mixture was degassed by purging with N_2 through a syringe needle for 30 min. The vessel was closed, sealed with Parafilm®, and allowed to slowly rotate for 24 h on an overhead shaker at RT. **CSP1** was collected by vacuum filtration through a sintered glass frit (DURAN®, porosity 4), washed with THF (200 mL), MeCN (100 mL), MeOH (100 mL), and distilled water (200 mL, two times each), and dried at 40 °C in a vacuum oven for two days. The modified silica particles were sieved through an analytical sieve (40 µm mesh) before being used in column packing. Yield: 2.67 g, 84 wt%.

Preparation of coated CS (CSP2)

The above **CS** (0.192 g) was dissolved in THF (40 mL) and the solution was transferred to a round-bottomed flask that contained **AzPS** (3.0 g; coating amount = 6.0 wt%). The mixture was sonicated for 20 min in an ultrasonic bath. THF was slowly evaporated at 40 °C and 357 mbar in a rotary evaporator. After evaporation and drying in a vacuum oven at 40 °C overnight, the coated silica particles were sieved through an analytical sieve (40 µm mesh) before being used in column packing. Yield: 2.79 g, 87 wt%.

Column packing

Each CSP (2.3 g) was immersed in a mixture of IPA (20 mL) and acetic acid (100 µL). The suspension was sonicated in an ultrasonic bath for 20 min to form a homogeneous slurry, which was then introduced under high pressure (max. 290 bar) into an empty stainless steel HPLC column in an *in-house* column-packing apparatus. MeOH was used (approx. 120 mL) as the compacting agent. After packing, the columns were rinsed with IPA in a standard HPLC setup (flow rate: 1.0 mL/min) and stored until further use.

HPLC method

Before use, the **CSP1** and **CSP2** columns were rinsed with *n*-hex/IPA using a stepwise gradient (30:70, 60:40, 90:10, v/v). The concentration of all analytes was 1 mg/mL. The flow rate and injection volume were set to 1 mL/min and 5 µL, respectively. The absorbance of all analytes was recorded at 254 nm. *p*-Cymene was used to determine the dead time (t_0) of the system. All measurements were carried out in triplicate.

Results and discussion

One-pot, three-step synthesis of the CS

Cellulose 3,5-dimethylphenyl carbamate containing propynyl carbamate substituents as a chemical anchor for subsequent *click chemical* immobilization to pre-functionalized **AzPS** was synthesized in three steps

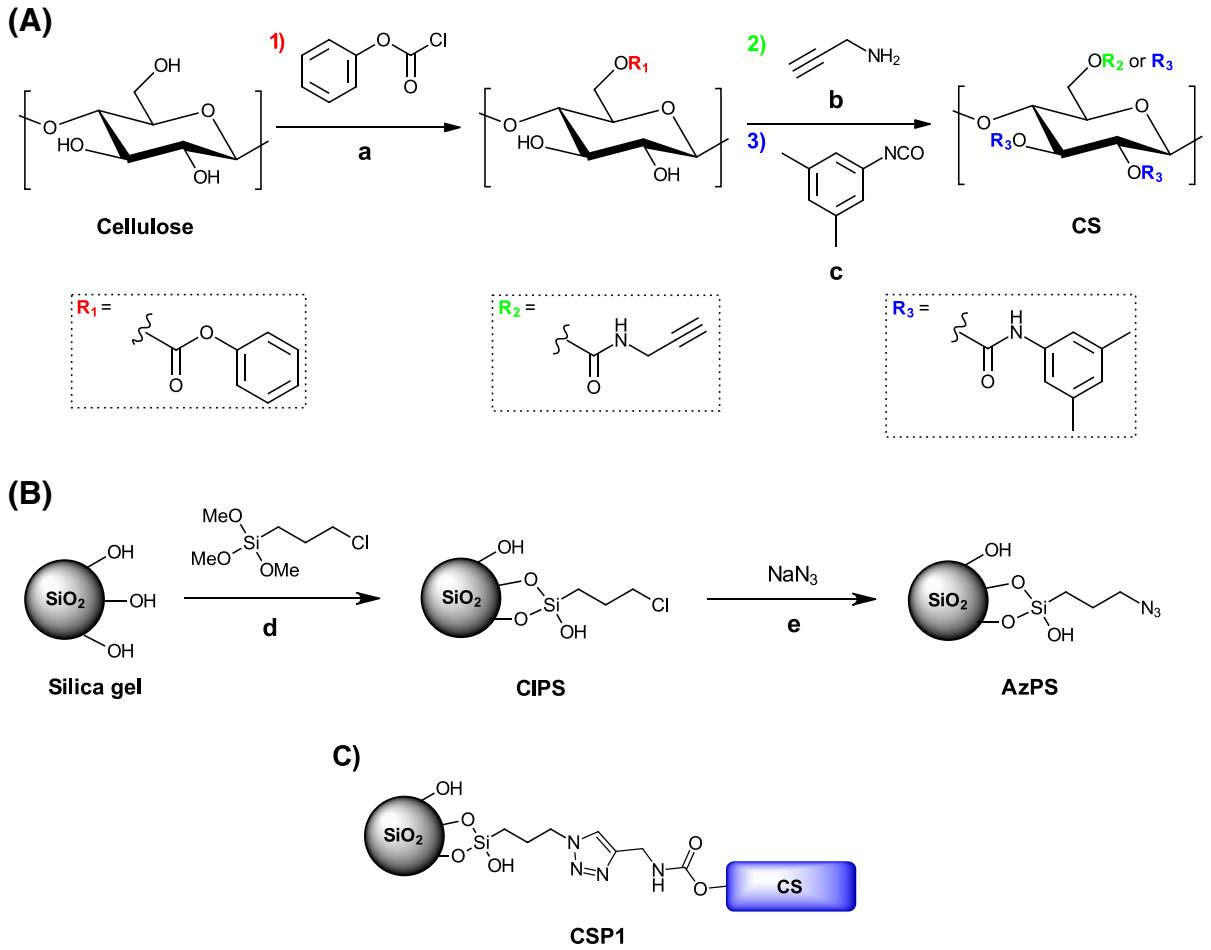


Fig. 1 (**A**) One-pot, three-step synthesis of the cellulose 3,5-dimethylphenyl carbamate-type **CS** carrying propynyl carbamate anchor groups; conditions: (a) DMAc/LiCl/pyridine, 0 °C to RT, N_2, 12 h; (b) DMAc/LiCl/pyridine/DMF, 40 °C, N_2, 12 h; (c) DMAc/LiCl/pyridine/DMF, 80 °C, N_2, 18 h; (**B**) pre-functionalization of silica gel for obtaining **AzPS** *via* **ClPS**; conditions: (d) toluene, *p*-toluenesulfonic acid (cat.), 80 °C, N_2, 48 h; (e) DMSO, tetra-*n*-butylammonium iodide (cat.), 80 °C, N_2, 72 h; (**C**) chemical structure of the 1,2,3-triazole linker after heterogeneous *click chemical* immobilization of the **CS** onto **AzPS** yielding **CSP1**

in one pot by applying both carbonate aminolysis and isocyanate chemistry (Fig. 1).

Cellulose was homogeneously dissolved in DMAc/LiCl according to a protocol by Heinze et al. (Pourjavadi et al. 2011; Elschner et al. 2013; Ganske and Heinze 2018). After the addition of pyridine, phenyl chloroformate was added (0.3 molar eq.). The higher reactivity and selectivity of phenyl chloroformate towards C6-OH *versus* C2-OH and C3-OH have already been reported (Elschner et al. 2014; Ganske and Heinze 2018). In a previous study, we have optimized the protocol towards quantitative oxycarbonylation of C6-OH (Bui et al. 2022b) and also reported on the optimization and successful aminolysis reaction between the polysaccharidic carbonates (Bui et al. 2022a). The obtained phenyl carbonate served as a reactive intermediate for subsequent aminolysis with propargylamine yielding a propynyl carbamate moiety as an alkyne donor for immobilization of the **CS** onto 3-azidopropyl-functionalized silica gel (Fig. 1C). After aminolysis, the residual OH groups were reacted directly, in the same pot, with 3,5-dimethylphenyl isocyanate for obtaining a "classical" cellulose 3,5-dimethylphenyl carbamate-type CS. The degrees of substitution of propynyl carbamate and 3,5-dimethylphenyl carbamate substituents

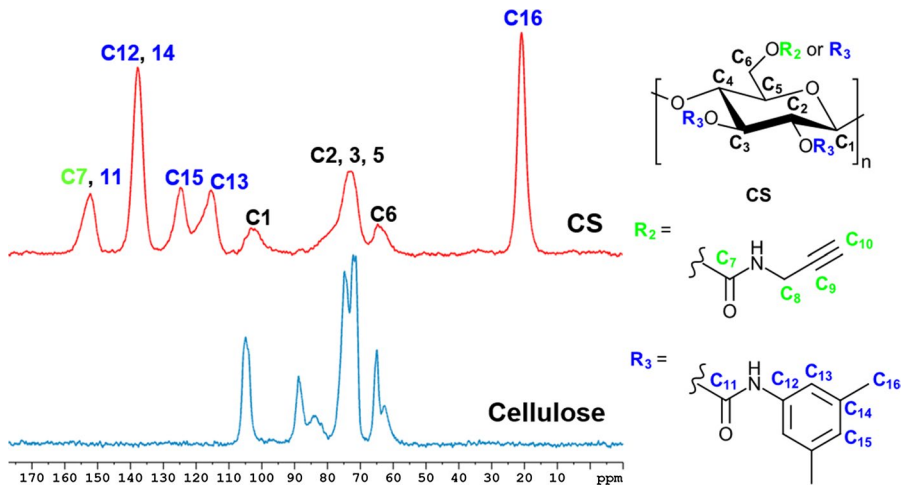


Fig. 2 Solid-state ^{13}C NMR spectra of **CS** (red) *versus* microcrystalline cellulose (blue)

Table 1 Elemental analysis results

Comp	Calculated (wt%)				Found (wt%, n = 3)				
	C	H	N	O	C	H	N	O	Cl
CS	65.66	6.18	6.96	21.20	63.38 ± 0.11	6.13 ± 0.23	6.57 ± 0.05	22.98 ± 0.57	–
ClPS	–	–	–	–	0.26 ± 0.00	< 0.05	< 0.05	–	0.17 ± 0.00
AzPS	–	–	–	–	0.21 ± 0.02	< 0.05	0.14 ± 0.00	–	< 0.01
CSP1	–	–	–	–	3.87 ± 0.01	0.40 ± 0.00	0.53 ± 0.00	–	–

Deviations from the calculated values are the result of incomplete substitution

calculated based on EA results (Supplementary Information) were 0.14 and 2.82, respectively. The yield of derivatization was 98.7%.

The FTIR, HSQC liquid-state NMR and solid-state ^{13}C NMR spectra of the **CS** are shown in Figs. S1, S2, and Fig. 2, respectively. The EA results for the **CS** are given in Table 1. In the FTIR spectrum of the **CS** (see Fig. S1), the bands assigned to N–H groups at 3321, C=O groups at 1720, C=C aromatic ring signals at 1611, 1539, 1450, 839, and C–O at 1212 cm^{-1} indicated that the carbamoylation reaction was successful, with the product fully matching the literature data of cellulose 3,5-dimethylphenyl carbamate-type selectors (Liu et al. 2013; Wei et al. 2019). In the solid-state ^{13}C NMR spectrum of **CS**, the resonances assigned to carbamate C=O groups at 152.6, C=C aromatic ring carbons at 138.3, 125.0, and 116.1, as well as CH_3-Ph at 20.8 ppm support the successful carbamoylation (Fig. 2).

The presence of resonances assigned to C8 at 2.7/31.9 ppm and C9 at 6.1/84.2 ppm ($^{1}H/^{13}C$, see the solution HSQC NMR spectra in Fig. S2) proved also the presence of propynyl groups on the polysaccharide backbone of the **CS**. Note that these resonances were not visible in the solid-state ^{13}C NMR spectrum due to the low propargyl carbamate DS. The molecular weight, dispersity (Đ), and calculated DP of **CS** were 210 kDa, 2.4, and 347, respectively.

Pre-modification of silica gel

3-Chloropropyl-functionalized silica gel (**ClPS**) was synthesized as an intermediate by condensation of (3-chloropropyl)trimethoxysilane with neat silica gel in toluene. The 3-chloropropyl-modified silica gel was subsequently converted to 3-azidopropyl-functionalized silica gel as the actual carrier material by nucleophilic substitution with NaN_3 in DMSO (Fig. 1B). The synthesis and the chemical structure of **ClPS** were evaluated by solid-state ^{29}Si and ^{13}C NMR. The EA results are summarized in Table 1. The different types of condensation of trialkoxysilanes onto silica gel and their resonances in solid-state ^{29}Si NMR according to (Salon et al. 2007)

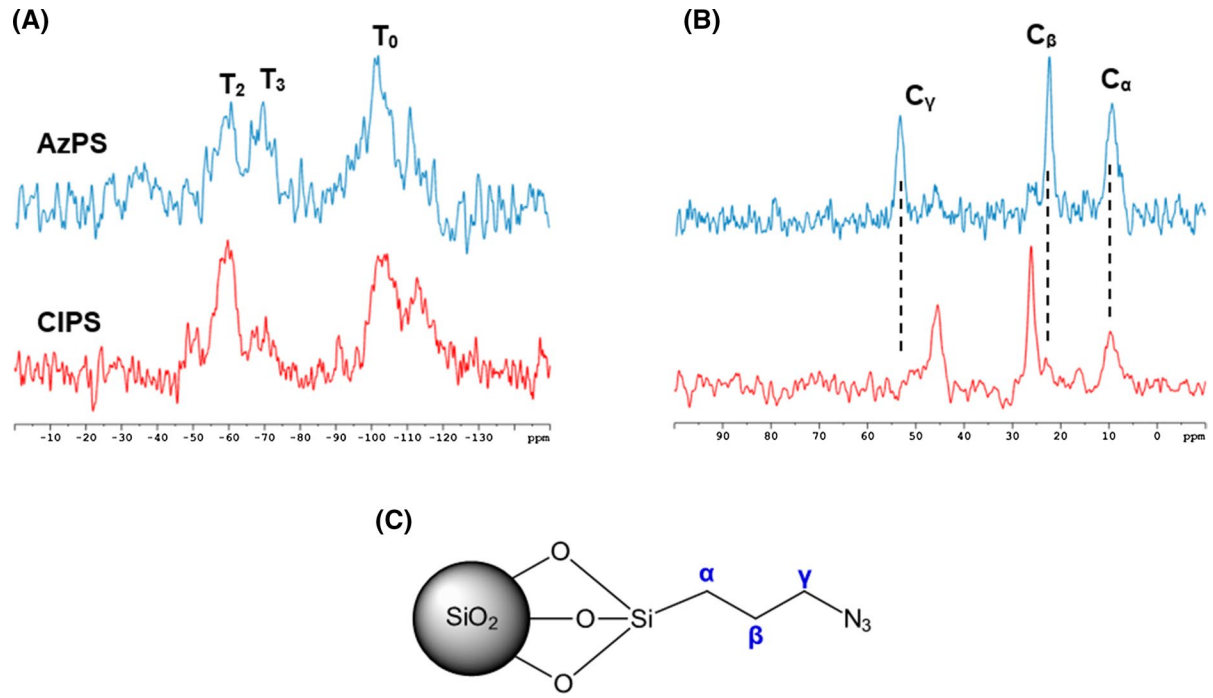


Fig. 3 **A)** Solid-state ^{29}Si and **B)** solid-state ^{13}C NMR spectra of **AzPS** (blue) *versus* **ClPS** (red), and **C)** the chemical structure of **AzPS**

are illustrated in Fig. S3. The solid-state ^{29}Si NMR spectra of **ClPS** *versus* neat silica gel and the solid-state ^{13}C NMR spectrum of **ClPS** are shown in Figs. S4 and S5, respectively. The additional resonances in the ^{29}Si NMR spectrum assigned to T_1 at approx. -50 ppm, T_2 at approx. -60 ppm, and T_3 at approx. -70 ppm and the resonances of C_γ at 45.5, C_β at 26.1, and C_α at 9.7 ppm in the ^{13}C NMR spectrum of **ClPS** indicated successful fixation of 3-chloropropyl groups on the silica matrix. The loading of 3-chloropropyl groups was calculated to be 0.43 wt% based on EA (corresponding to 48 µmol/g silica).

The chemical structure of **AzPS** was characterized by the above-mentioned solid-state NMR methods as well (Fig. 3). In addition, rhodamine B propargyl ester was used as a color indicator to further confirm the presence of azido-groups and their reactivity in *click* reactions (see Figs. S6 and S7).

The solid-state ^{29}Si NMR spectrum of **AzPS** showed resonances assigned to T_2 structures at -60 ppm and T_3 at -70 ppm, and resonances of C_γ at 53.4 (45.5 ppm for **ClPS**), C_β at 22.4 (26.1 ppm for **ClPS**), and C_α at 9.3 ppm in the ^{13}C NMR spectrum. In addition, the purple-to-pink color of the azido-modified silica gel "*clicked*" with rhodamine B propargyl ester further proved the presence of azido groups in a qualitative manner. The color test was used as an indirect proof since in ATR-FTIR no azide bands were visible due to their low concentration (33 µmol/g based on an N content of EA).

Click immobilization of CS onto AzPS

The obtained **CS** carrying propynyl carbamate groups at a low level of substitution (DS = 0.14) was chemically linked to pre-modified silica gel by Cu(I)-catalyzed *Huisgen* alkyne-azide cycloaddition under mild conditions. The loading amount of immobilized **CS** on **CSP1** was determined by EA (see Table 1) and TGA (see Fig. S8). The total organic content of **CSP1** based on EA was 6 wt% (O content estimated based on the oxygen content of the selector). The organic contents of pure silica gel, **AzPS**, and **CSP1** based on the mass change by TGA were approx. 0.6, 1.1, and 7.0 wt%, respectively (see Fig. S8). Thus, the results of both methods agreed very well. To increase the loading amount of **CS** on the silica gel, we tried to increase the anchor points on both **CS** (more

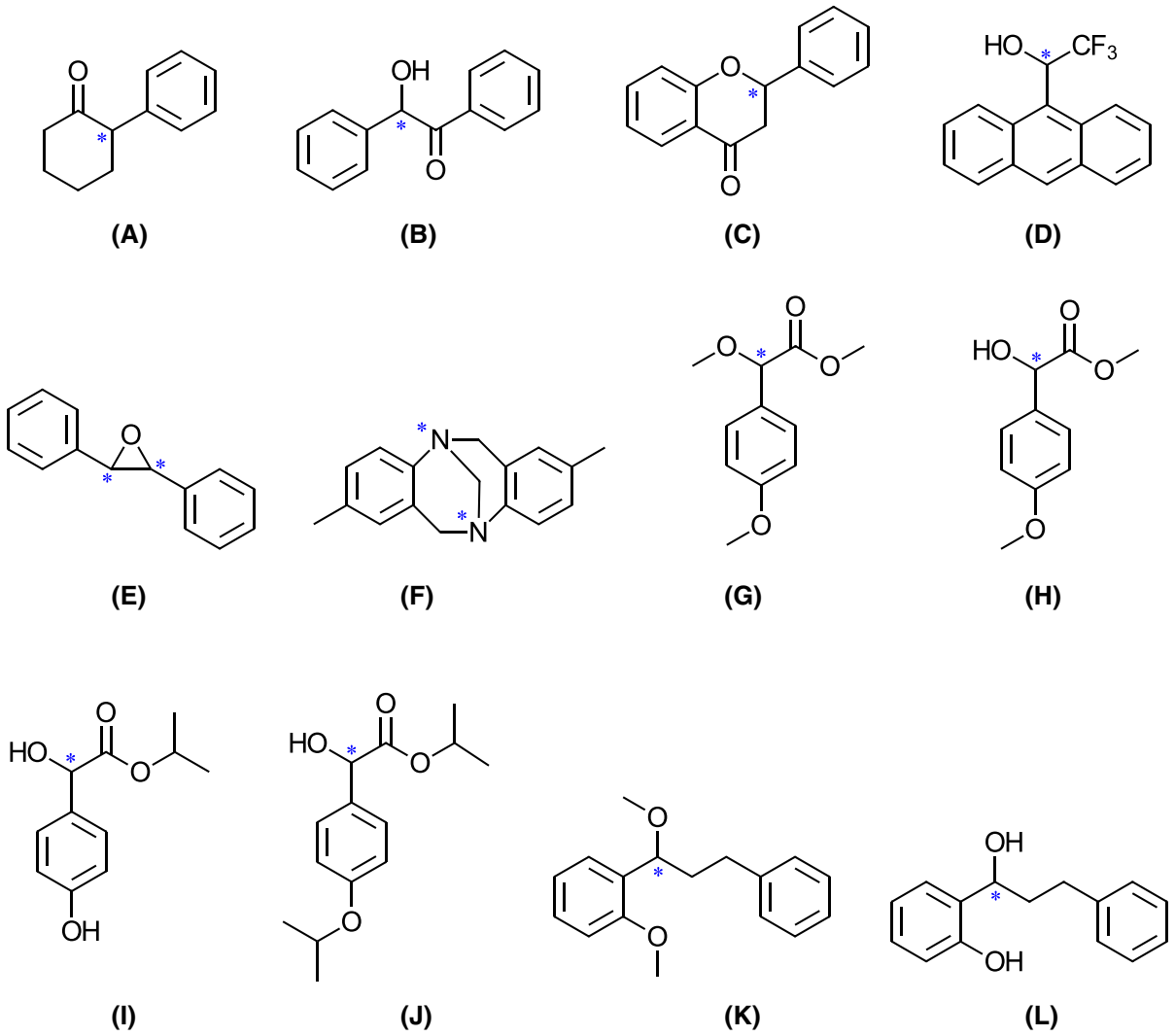


Fig. 4 The chemical structures of the chiral analytes **a**-**l**

propargyl groups) and **AzPS** (higher azide loading), however, the organic content of the corresponding CSP still did not exceed 7.4 wt% (TGA). Due to the low surface area of the silica gel used in this study (7 µm particle size, 1000 Å pore size, 25 m^2/g surface area), it thus seemed difficult to achieve a higher loading degree. Higher loading amounts can be achieved using silica gel with a higher surface area (ongoing work).

For comparative purposes, **AzPS** was also physically coated with **CS** with the same loading amount (6 wt%).

Enantioseparation evaluation

Twelve chiral analytes (2-phenyl cyclohexanone (**a**), benzoin (**b**), flavanone (**c**), Pirkle's alcohol (**d**), *trans*-stilbene oxide (**e**), Tröger's base (**f**), four mandelic acid derivatives (**g**-**j**), 1-methoxy-2-(1-methoxy-3-phenylpropyl) benzene (**k**), and 1-(*o*-hydroxyphenyl)-3-phenyl-1-propanol (**l**), see Fig. 4) were used in this work to study the enantioseparation performance. We compared the immobilized selector **CSP1** and the coated-only alternative **CSP2**, both with a **CS** loading of 6 wt% and the same pre-modified silica gel for ensuring a fair

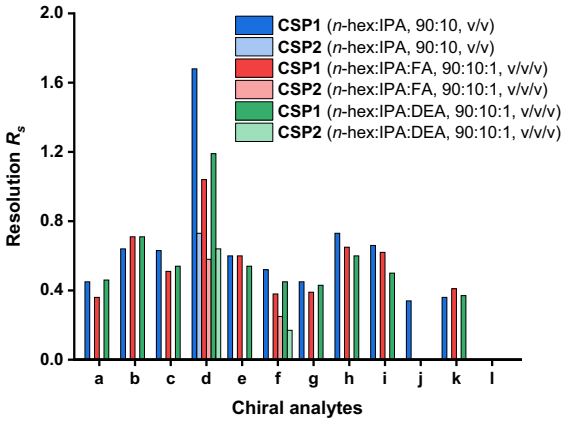


Fig. 5 Resolution (R_s) values of the chiral analytes on **CSP1** and **CSP2** with different mobile phases: *n*-hex/IPA (90:10, v/v); *n*-hex/IPA/FA (90:10:1, v/v/v); and *n*-hex/IPA/DEA (90:10:1, v/v/v)

comparison. A "standard" mobile phase composed of *n*-hexane and IPA (9:1, v/v) was used. Furthermore, also "non-standard" mobile phases containing THF and $CHCl_3$ were employed to study the enantioseparation performance and evaluate the stability of the immobilized-type **CSP1**. The reproducibility of the enantioseparation performance of **CSP1** had been examined once per month over five months. The material proved to be perfectly stable. There was only a minute difference found (*e.g.*, RSD = 3.5% for compound **e** in *n*-hex:IPA:$CHCl_3$, 87:10:3, v/v/v). Before the tests, **CSP1** had been additionally rinsed with a large excess of THF (in which the selector is soluble), MeCN, MeOH and water to ensure that only covalently linked selector is left at the CSP and any contributions of adsorbed **CS** can be excluded.

Different mobile phases were used for the comparative studies: *n*-hex/IPA (90:10, v/v), *n*-hex/IPA/FA (90:10:1, v/v/v), and *n*-hex/IPA/DEA (90:10:1, v/v/v). The chromatographic retention factor (k_1), selectivity (α), and resolution (R_s) of the twelve chiral analytes on the two CSPs are summarized in Tables S1–3. Figure 5 shows the resolution (R_s) values of the chiral analytes on **CSP1** and **CSP2**. Representative HPLC chromatograms of the analytes **b** and **d** are shown in Fig. 6. In the "standard" solvent *n*-hex/IPA (90:10, v/v), all of the selected chiral analytes were at least partially separated on **CSP1**, except racemate **l**. The chiral analyte **d** was the only baseline-separated compound on **CSP1** with $R_s = 1.68$ and $\alpha = 1.93$. On the other hand, none of the selected chiral analytes were separated on **CSP2** using this solvent mixture, except chiral analyte **d**, which was at least partially separated with $R_s = 0.73$ and $\alpha = 1.86$. This demonstrated a clear performance advantage of **CSP1** over **CSP2** at this comparably low overall selector loading.

The addition of acidic and basic modifiers to the mobile phase (FA or DEA, each 1.0 vol%) slightly changed the separation performance. With the two new mobile phases (*n*-hex:IPA:FA, 90:10:1, v/v/v; and *n*-hex:IPA:DEA, 90:10:1, v/v/v), all of the selected chiral analytes were partially separated on **CSP1** except **j** and **l**, while the two analytes **d** and **f** could now be partially separated on **CSP2**. The enantioseparation performance of **CSP1** was generally higher than the one of **CSP2**. The reasons for this are not yet clear. Different geometries and spatial alignments of the **CS** during the coating and immobilization process could lead to those differences in the enantioseparation performance between the two CSPs.

Further studies with smaller silica particles and higher surface areas, allowing higher efficiencies and consequently higher selector loadings with higher retention and selectivity, are ongoing. A previous comparison from our work (Bui et al. 2022b) with coated materials carrying 20 wt% of selector has shown that the overall performance matched that of commercial reference materials. However, with the present silica dimensions (*i.e.*, relatively low surface area), it was not possible to achieve such high selector loadings (*vide supra*). It was all the more surprising that a generally higher enantioseparation performance was observed in the case of the immobilized-type CSP *versus* the coated-type one, which is quite an encouragement for further studies of this immobilization method.

Two categories of "non-standard" mobile phases—with strong eluents, such as THF and $CHCl_3$—at different ratios were selected to study the influence of eluent modification on the enantioseparation performance as well as to evaluate the stability of **CSP1**. We intended to demonstrate that it is possible to run the chromatographic system also with mobile phases that would dissolve the **CS** in coated-only column fillings. This applies especially to THF, which was used for dissolution and immobilization during material preparation. The respective chromatographic data are summarized in Tables S4–5. Representative

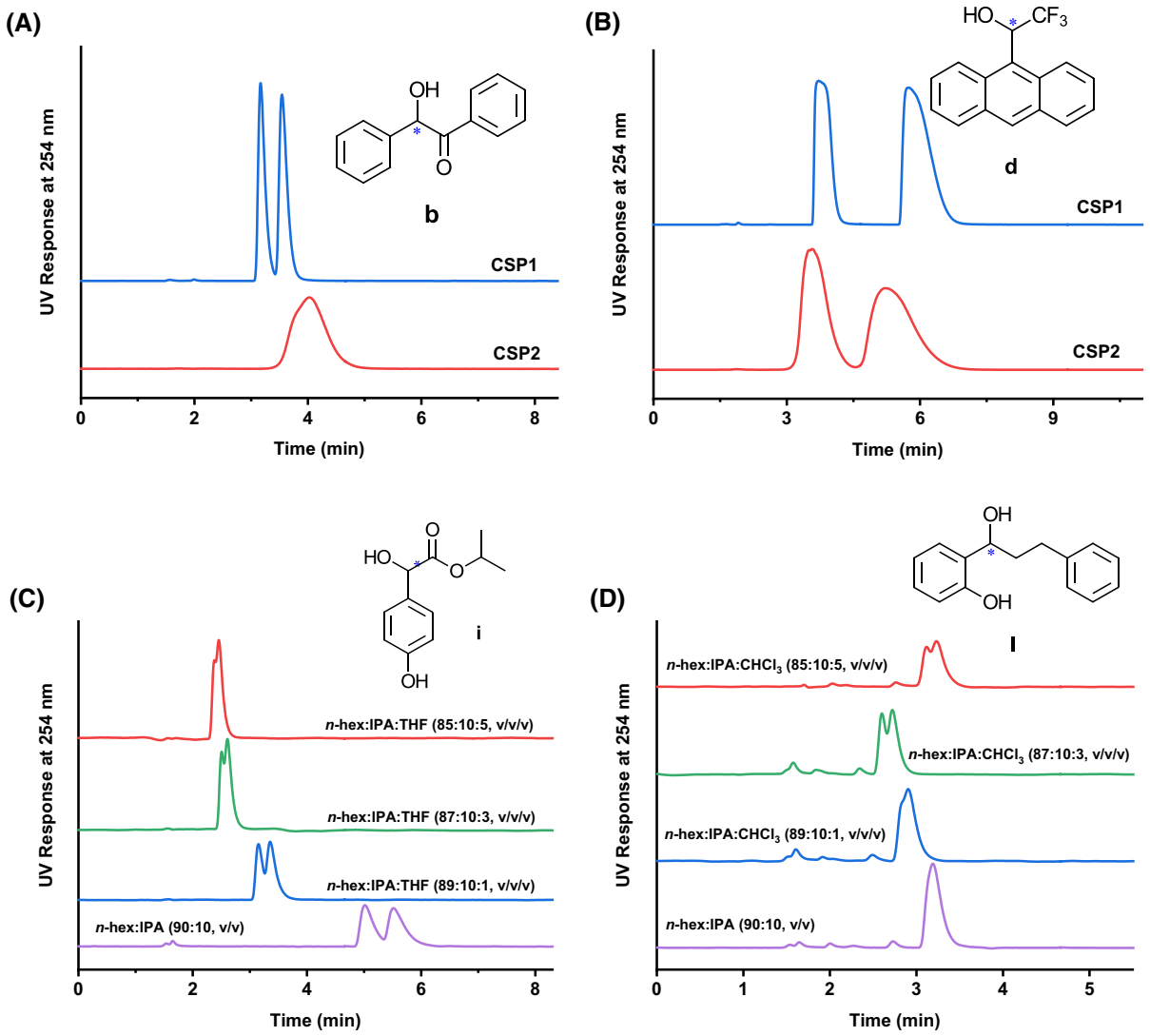


Fig. 6 HPLC chromatogram of (**A**) chiral analyte **b** and (**B**) compound **d** on **CSP1** and **CSP2** using *n*-hex:IPA (90:10, v/v) as the mobile phase; (**C**) chiral analyte **i** on **CSP1** with *n*-hex:IPA:THF at different ratios as the mobile phases; (**D**) chiral analyte **l** on **CSP1** with *n*-hex:IPA:$CHCl_3$ at different ratios as the mobile phases

HPLC chromatograms of chiral analyte **i** using *n*-hex:IPA:THF (at different ratios) and chiral analyte **l** using *n*-hex:IPA:$CHCl_3$ (at different ratios) as the mobile phases are shown in Fig. 6C, D. In Fig. 7, the respective R_s values on **CSP1** are displayed.

Increasing the percentage of THF in the mobile phase generally reduced the R_s of the chiral analytes on **CSP1**. The significant influence of THF can be nicely illustrated by means of chiral analyte **d**. It was baseline separated on **CSP1** with *n*-hex:IPA (90:10, v/v, $R_s = 1.68$), but only partially separated at higher THF contents ($R_s = 1.33$, 1.43, and 0.92, corresponding to 1, 3, and 5% of THF in the mobile phase, see also Table S4). The effect of $CHCl_3$ on the separation of the selected chiral analytes was not always following such a general trend. Compound **d** was baseline separated on **CSP1** using the "standard" eluent *n*-hex:IPA (90:10, v/v, $R_s = 1.68$). The resolution increased to 1.96 when 1% of $CHCl_3$ was added, but decreased again when the percentage of $CHCl_3$ was further increased (see Fig. 7B and Table S5). The chiral analyte **l** could not be separated on **CSP1**

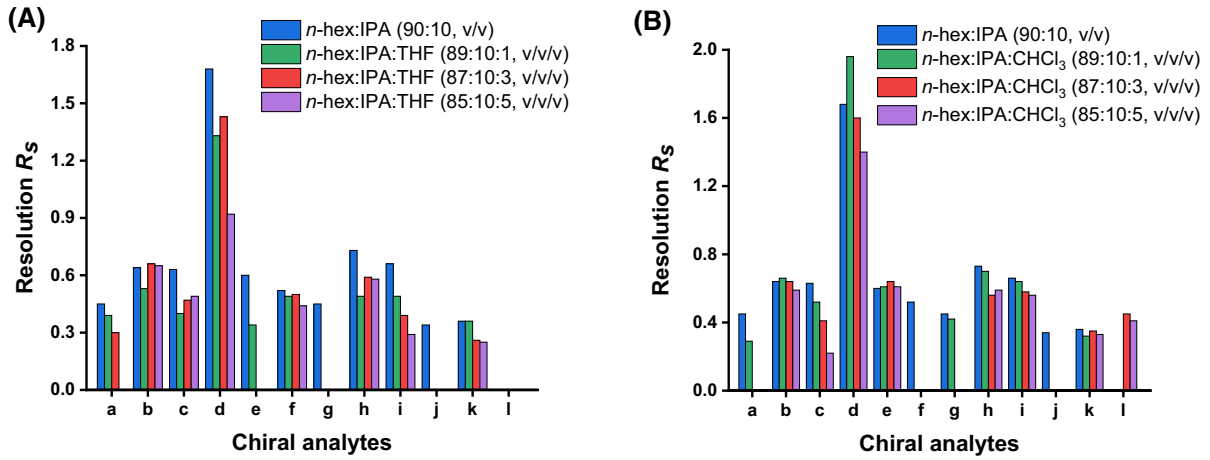


Fig. 7 Resolution (R_s) of the chiral analytes on **CSP1** using different mobile phases: (**A**) with THF as a mobile phase component; (**B**) with chloroform as a mobile phase component

with *n*-hex:IPA (90:10, v/v) and *n*-hex:IPA:$CHCl_3$ (89:10:1, v/v/). However, when 3 or 5% of $CHCl_3$ were added, the selectivity increased and the compound was partially separated ($R_s = 0.45$ and 0.41, respectively).

Conclusions and outlook

Aminolysis of cellulose carbonates and isocyanate chemistry were optimized, and a three-step, one-pot procedure was employed to synthesize a cellulose 3,5-dimethylphenyl carbamate-type chiral selector carrying propynyl carbamate functional groups (DS approx. 0.14). The compounds' structures were confirmed by both FTIR spectroscopy, solid-state, and solution-state NMR spectroscopy. Additionally, the chiral selector was characterized by GPC and elemental analysis. In parallel, the synthesis of a modified silica gel carrier (3-azidopropyl-functionalized silica gel) *via* a 3-chloropropyl intermediate was carried out. The pre-functionalized silica gel matrices were characterized by both solid-state ^{29}Si and ^{13}C NMR as well as EA.

The **CS** carrying alkyne moieties was immobilized on 3-azidopropyl-functionalized silica gel by Cu(I)-catalyzed alkyne-azide cycloaddition *click* chemistry, in which a selector loading of 6 wt% was achieved. Attempts to increase the loading amount by increasing the number of anchor points (alkyne-groups) on the CS and azido-groups on silica gel were made but were unsuccessful given the low accessible surface area of the silica material. With different silica particle characteristics (especially silica materials with higher surface area), loading amounts comparable to commercially used 20 wt% can be achieved also with this mild immobilization protocol as we will demonstrate in follow-up communications.

The enantioseparation performance of the immobilized CSP was evaluated by using twelve chiral analytes and different eluents, and was compared to a coated-type CSP of the same silica material, selector quality and quantity. The immobilized CSP generally performed better than its coated counterpart. The reasons for this—somewhat unexpected but satisfying—finding need to be further studied in more detail, especially by using silica particles with higher surface area and a selector loading in the range of 20 wt%.

In this contribution, we present a new strategy for the synthesis of chiral selectors by a combination of oxycarbonylation/aminolysis and isocyanate chemistry, as well as the stable and robust immobilization of respective alkyne-carrying carbamate-type selectors onto silica by a mild *click chemical* protocol and demonstrate the promising performance characteristics of this immobilized CSP in enantioseparation.

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Author contributions C.V.B, T.R. and H.H. contributed to the study conception and design. Material preparation, data collection, and analysis were performed by C.V.B. The original

draft of the manuscript was written by C.V.B., including visualization. Review & editing by T.R. and H.H. Supervision, project administration and funding acquisition by T.R. and H.H. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Consent for publication All authors agreed to the publication in the submitted form.

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